

Effect of Vaccination against *Mycoplasma hyopneumoniae* in a Pig Herd from Birth to Slaughter

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Abstract

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Mycoplasma hyopneumoniae remains an important pathogen in swine industry. The aim of the present work was to assess *M. hyopneumoniae* vaccine from viewpoint of antibodies formation, health, daily weight gain, quality of meat and lung lesions. The investigation was carried out on forty four cross-bred 7-day-old piglets free from *M. hyopneumoniae* infection. Twenty two piglets were vaccinated intramuscularly. The other group of twenty two piglets was used as a control (unvaccinated). The results showed that in both groups the antibodies against *Mycoplasma hyopneumoniae* were not formed before vaccination. Antibodies against enzootic pneumonia in the vaccinated group were formed after fourteen days from the second vaccination and remained present until day 77. It was observed that in unvaccinated group antibodies against *M. hyopneumoniae* had not been formed. Pigs with artificially acquired active immunity from vaccination achieved higher daily weight gain, growth rate, fattening and finishing weight, improved quality of meat and decreased pneumonia lesions than the pigs in the control group.

Enzootic pneumonia, antibodies, immune, response

Mycoplasma hyopneumoniae is the primary agent of enzootic pneumonia in pigs. The disease is characterized by a chronic nonproductive cough, retarded growth rate, and inefficient utilization of feed (Ross 1992; Kobish et al. 1993).

Segregated early weaning has been reasonably successful in minimizing infection levels in pigs, yet *M. hyopneumoniae* vaccinations are a prudent control measure for some herds with animals in good health. Repopulation of herds with *M. hyopneumoniae*-free pigs is not widely applied because it is expensive (Stärk et al. 1992). The principal weapons in this are correct handling, medicine-based prevention and immunity prophylaxis by vaccination. Natural outbreaks of mycoplasma pneumonia are almost always accompanied by a variety of secondary bacterial infections and it is almost impossible to stimulate these infections under experimental conditions (Morrison et al. 1985). For this reason it is important to evaluate them under practicable condition, even if the vaccines show outstanding promise based upon laboratory data. Some field trials demonstrated that *M. hyopneumoniae* vaccines, based on adjuvanted whole cells, turn out to confer beneficial effects in one or a few herds clinically infected by enzootic pneumonia (Charlier et al. 1994; Dohoo et al. 1996). In addition to being efficacious and safe, the vaccine, destined to be used in animals of finite worth, should also be cost-effective (Walker 1992). Lesions typical of enzootic pneumonia occur in 30% to 80% of slaughter pigs (Ross et al. 1993). *M. hyopneumoniae* bacterins have been useful in reducing lung lesions and improving growth performance in endemic herds (Maes et al. 1999).

Materials and Methods

Forty four cross-bred 7-day-old piglets from one Lithuanian farm were randomly split into groups of males and females. Piglets were free from *M. hyopneumoniae* infection. Twenty two (11 females and 11 males) of 7-day-old piglets were vaccinated against *M. hyopneumoniae* with commercially available vaccine Respisure (Pfizer AH,

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U.S.A.) of 2 ml behind the ear, as recommended by vaccine protocol. The first dose was administered during the first week of life. After 14 days the second vaccination followed at the same vaccine doses. The other group of 22 piglets (11 males and 11 females at the age of 7 days) was used as control (unvaccinated) group. Blood samples of both groups of piglets were taken before vaccination and on days 21, 35, 49, 63, 77 after vaccination.

Concentration of *M. hyopneumoniae* specific antibodies in serum was determined by blocking ELISA (Dako, Denmark). The serum samples were diluted 1:10 and incubated for 1 1/2 h in microwells precoated with *M. hyopneumoniae* antigen. Then without emptying the wells, peroxidase-conjugated mouse monoclonal antibody to a *M. hyopneumoniae* – specific epitope on 74 kDa protein was added. The higher the antibody concentration in a pig serum was, the less monoclonal antibody conjugate was bound to the well. After 15 min of incubation the microwells were washed and chromogenic substrate (1.2 phenylenediamine dihydrochloride) was added. A golden-brown colour developed in all wells, and after 10 min the reaction was stopped by the addition of sulphuric acid. The higher the antibody concentration in the pig serum specimen was, the lower the intensity of the colour was detected in the well. The absorbance (OD) in each microwell was read at 490 nm and the absorbance of specimen wells was compared with the absorbance of a buffer control well. Positive specimens gave OD - values, which were less than 50 of the OD - value of the buffer control well.

The vaccinated and control animals were housed separately during the weaning and thereafter. During the fattening period they were kept in the same air space but were separated by a door. At the age of 33 days the piglets were transferred to the post-weaning unit. They were weighed individually at each move between units and subsequently to slaughter.

Prevention measures (castration, iron injection, needle teeth clipping, tail docking) and other management practices were identical for both groups.

Vaccinated and control groups were compared during weaning/growing/finishing period. Live body weight was measured at the age of 7, 33, 107 and 206 days. Daily weight gain (DWG) in each group was calculated as the difference between mean weight at the start and at the end of finishing period divided by the number of fattening days of a group.

Back fat thickness, muscle thickness and lean meat content were recorded by ultrasound PIGLOG 105 measurement (SFK - Technology, Denmark) before slaughter. The measurement was performed at two predetermined anatomical sites: fat 1 between 3rd and 4th last lumbar vertebrae (7 cm from midline) fat 2 and muscle thickness between 3rd and 4th last rib (10 cm) and 7 cm from midline.

The lungs of vaccinated and unvaccinated groups of pigs were examined at slaughter. The same person determined the incidence of macroscopic lesions. Lung lesion was scored by percentage (Goodwin et al. 1968). The extent of lung lesions was recorded onto a lung diagram. Surface areas showing pneumonia for each lobe were given a score from 1 to 5. Total score by per cent is 55. This consist from left apical lobe 10%, right apical lobe 10%, left cardiac lobe 10%, right cardiac lobe 10%, cranial edge of left diaphragmatic lobe 5%, cranial edge of right diaphragmatic lobe 5% and for intermediate lobe 5%. The lung lesions were also evaluated by score method. The maximum score for each lobe was 5 and for the all lungs 35 (Hannan et al. 1984).

Percentage of reduction in lung lesion scores relative to control group was calculated for the vaccinated group by formula - mean control group minus vaccinated group multiply by 100 and divided to mean control group.

Lungs with gross lesions were selected for microbiology investigations. All mycoplasma cultivation procedures were performed according to the methods used by the Mycoplasma Section at the Danish Veterinary Laboratory, Copenhagen (Friis 1974, 1975). For mycoplasma isolation, tissue was homogenized in tissue grinder using 5 ml of Friis medium. Lungs suspensions were inoculated in 10 – 100 000 fold dilutions in Friis medium. Inoculation was carried out at +35 – 37 °C in a roller drum. Cultures with acid shift were subcultured 3 - 5 times and inoculated on Friis agar. Isolated strains of mycoplasma were identified by the disc growth inhibition test (DGI), using antisera against the type “J” strain of *M. hyopneumoniae* and strain Ms 42 of *Mycoplasma flocculare* (*M. flocculare*).

Statistical analyses were performed using Microsoft Excel software. Significance was set at $P < 0.05$.

Results

The antibodies against *M. hyopneumoniae* were not detected before immunisation in both groups. The mean OD - value of vaccinated and control groups were 119.52% and 119.12%, respectively.

Antibodies against *M. hyopneumoniae* in the vaccinated group had been formed on 14 days after the second vaccination. OD - value of positive pigs in vaccinated and unvaccinated groups was 14.24% and 90.21%, respectively. Concentration of antibodies in vaccinated group remained till 77 days (OD - value 46.6). In control group all sera samples were seronegative - OD - value 128.48%. Table 1 shows the presence of antibodies against *M. hyopneumoniae*.

The DWG increased by 28 grams (g) in the vaccinated group compared to that of the control group. The growing body weight of the vaccinated group was significantly greater by 9.55 kg ($P < 0.001$) than that of control group (Table 2).

Table 1
Mycoplasma hyopneumoniae antibodies in pigs aged 7- 77 days

Pigs groups	Results	7 day	21 day	35 day	49 day	63 day	77 day
Vaccinated	Positive			22	22	19	22
	Dubious					4	
	Negative	22	22				
Control	Positive						
	Dubious						
	Negative	22	21	19	20	21	19

Table 2
Effect vaccine on some production indices of pigs

Indices	Groups of pigs	
	Control	Vaccinated
Daily weight gain DWG (kg /day)	0. 492	0. 520
Initial weight (kg)	2. 971	2. 915
Weight (kg) after weaning	9. 623	9. 782
Weight (kg) at start of the fattening	46. 230	55. 780
Finishing weight (kg)	104. 2	110.10

In vaccinated group the measurements of fat 1 and fat 2 were less by 0.82 mm and 1.39 mm, respectively. Muscles in vaccinated group were bigger by 1.55 mm, the percentage of lean meat was larger in vaccinated group (Table 3).

Significantly larger proportion of lung surface with pneumonic lesions was detected among the unvaccinated pigs. Lower percentage rate of the lungs belonging to the vaccinated pigs had pneumonic lesions, indicating the efficiency of the used vaccine. Evaluation of lung lesions was made using two methods (Table 4).

M. hyopneumoniae was not isolated from lung specimens collected from vaccinated pigs. However, it was cultured from 5 out of 22 lungs (22.73) of the unvaccinated pigs.

Table 3
Effect of vaccination on quality of meat

Pigs groups	Fat 1 mm	Fat 2 Mm	Muscle Mm	Lean meat %
Vaccinated	15. 45	15. 74	51. 95	55. 01
Control	16. 27	17. 13	50. 4	54. 3

Table 4
Results of investigated lung surface lesions

Group of pigs	Goodwin method by percentage (%)	Hannan method by score
Vaccinated	3. 25	1. 871
Control	9. 00	3. 092

Discussion

A commercial *M. hyopneumoniae* bacterin induced protection against mycoplasmal pneumonia in pigs (Diekman et al. 1999; Maes et al. 1999; Thacker et al. 2000). According to the manufacturer guidelines, the piglets received the first dose at about 1 week

of age and the second dose after 2 weeks. Piglets younger than 3 days of age were not vaccinated because it is unclear whether the immune response in the pigs is optimal (Hammerberg et al. 1989). In the vaccinated group antibodies were detected 14 days after the second vaccination. In this group concentrations of specific serum antibodies were detectable on day 77. In unvaccinated pig group, *M. hyopneumoniae* specific antibodies had been not formed and enzootic pneumonia infection occurred. The DWG, one of the most important biological index, showed significant difference (28 g) in vaccinated pigs. The weight gain was lower than that observed (40 g) in field trials with Stellamune Mycoplasma (Charlier et al. 1994). In our studies the weight of vaccinated pig group increased during growing/fattening/finishing period.

Percentage reduction in lung lesions in the vaccinated group reached 66% by Goodwin and 57.4% by Hannan. *M. hyopneumoniae* was isolated from lungs specimens collected from unvaccinated pigs (22.73%). Ross et al. (1992) described more extended typical lung lesions (30% to 80%) of slaughter pigs. The lean meat, muscle thickness was greater in vaccinated group.

In conclusion, the results of our study indicate that pigs with artificially acquired active immunity had higher average daily weight gains, improved meat quality and reduced pneumonia lesions than the pigs in the non-vaccinated control group.

Sledování účinku vakcinace proti *Mycoplasma hyopneumoniae* u prasat od narození do porážky

Mycoplasma hyopneumoniae zůstává i nadále pro velkochovy prasat závažným patogenem. Cílem předkládané práce bylo zhodnotit účinek vakcinace proti *M. hyopneumoniae* z hlediska tvorby protilátek, zdraví zvířat, jejich denních přírůstků na váze, kvality masa a množství zjištěných plicních lézí. Naše sledování byla prováděna ve skupině čtyřiceti čtyř 7denních selat - kříženců, prostých infekce *M. hyopneumoniae*. Intramuskulárně bylo vakcinováno dvacet dva selat. Zbývajících dvacet dva selat sloužilo jako kontrolní nevakcinovaná skupina. Z dosažených výsledků vyplývá, že ani v jedné skupině sledovaných selat nedošlo před vakcinací k tvorbě protilátek proti *M. hyopneumoniae*. Specifické protilátky proti enzootické pneumonii prasat byly ve skupině vakcinovaných selat zjištěny za čtrnáct dnů po druhé vakcinaci a přetrvávaly až do 77. dne po vakcinaci. Ve skupině nevakcinovaných selat protilátky proti *M. hyopneumoniae* detekovány nebyly. Selata s aktivní imunitou, navozenou uvedenou vakcinací, dosahovala vyšších denních přírůstků na váze, rychlejšího růstu, vyšší porážkové váhy, vyšší kvality masa a nižších počtů plicních lézí, ve srovnání se selaty z kontrolní nevakcinované skupiny.

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